# PART V. VIRUS AND FUNGUS DISEASES

*Thomas Francis, Jr., Chairman*

## AIRBORNE Q FEVER

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In some of the literature from the National Research Council pertaining to this Conference it was stated among other things that interest should be directed toward how "the behavior of inhaled infective agents is related to infection." For Q fever the only known important natural method of initiation of disease in man is by inhalation of the causative agent, *Coxiella burnetii*. However, man-to-man transmission has been recorded only rarely. This capability to infect both man and animals by the respiratory route has been studied by almost every investigator who has examined this organism, many times inadvertently and less often by intent.

From the considerable body of information available about this infection in man and in animals, it would have been easy to assemble confirmed information. Such a compilation would probably have been neither speculative nor provocative and both terms were employed by those who defined the objectives of this meeting.

From a series of studies (5, 23, 24) conducted some years ago in association with a large group of colleagues, data have been selected which permit a comparison of this disease initiated in three systems: (i) in guinea pigs by intraperitoneal injection, (ii) in guinea pigs by inhalation, and (iii) in man by inhalation.

By comparing these three systems over a wide dose range, certain inferences will be drawn as to how the behavior of inhaled rickettsiae is related to infection. The AD strain of *C. burnetii*, an isolate from milk in California, was used (13). It had been through three guinea pig and eight egg passages. The material employed in this study was a single lot of whole egg slurry maintained in the frozen state. On the basis of infection, as detected by the complement fixation (CF) response of convalescent standard inbred guinea pigs inoculated intraperitoneally, the egg slurry routinely produced a 50% response at a dilution of $10^{-10.5}$. The inoculum per animal was 1 ml appropriately diluted with water. Above a $10^{-9}$ dilution of slurry, or in the zone between 1 and perhaps 150 infectious units, guinea pigs seldom become febrile. With an increase in dosage all guinea pigs regularly showed fever. Deaths were seldom seen until a $10^{-3}$ dilution was reached, and the $LD_{50}$ was usually reached at the $10^{-2}$ dilution.

To examine more closely the response of groups of animals around the infectious end point, we resorted to intraperitoneal inoculation of serial 2-fold dilutions of slurry beginning at $10^{-9.0}$, the observed 100% response level. Twenty animals were employed at each dilution. The CF results are shown in Fig. 1. The observed positive responses at each dilution are shown by the crossmarks, whereas the solid line describes the response predicted by Poisson's law of small numbers on the basis that one infecting particle per animal is capable of initiating disease as manifested by a serological response. It will be observed that, within the limits placed by having only 20 animals per group, the agreement is quite close. Based on this sort of titration, the most probable number (17) of infectious particles in the original slurry was some 20 billion per milliliter. A value of the same order was obtained by the less accurate method of direct counting.

The next step was to determine if a similar relationship existed when guinea pigs were exposed by the respiratory route. If, in fact, such a percental response difference did occur, it would provide evidence for a parity between the two routes, a point of scientific interest; but of more immediate concern, it would provide a direct method of accurate dose measurement for aerosols containing very small numbers of organisms, avoiding the errors inherent in any mechanical sampling system.

The methods of exposure utilized certain of the equipment which has been described previously (27). Aerosols composed primarily of particles in the 1-$\mu$ diameter range were produced from a

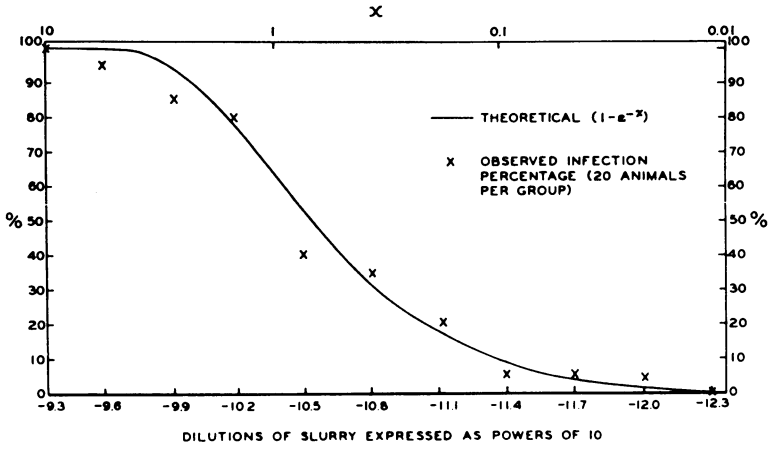


FIG. 1. *End point titration of Coxiella burnetii in guinea pigs. Values of* $\chi$ *per dose per animal on the hypothesis that a single particle can produce infection (i.e., serological response).*

standard generating device having a fixed original volume. The aerosol generated was allowed to equilibrate in a large sphere of known air volume which, of course, introduced a further dilution factor. Standard weight animals were exposed for 1 min, beginning the exposure when the cloud was 2 min old. Dose variations were introduced by appropriate dilution of the original slurry with noninfected egg slurry prior to aerosolization. The physical characteristics of the two slurries were similar so that the properties of the aerosol formed did not change appreciably regardless of the relative proportions of infected and noninfected slurries. After exposure the animals were held for 1 month and the CF status at that time was determined.

The results of replicate respiratory exposure trials are shown in Table 1. No animals were infected when exposed for a period of 1 min to the dilute aerosol created from a $10^{-6}$ dilution of the source slurry. When the original slurry was 10-fold more concentrated ($10^{-5}$), 1 of 26 animals in two trials had a positive CF reaction 1 month after exposure. At $10^{-4}$ dilution, 16% of the animals exposed were infected, and with a 10-fold more concentrated source strength, 84% were found positive. With less dilute material all animals developed antibodies. Thus a 10-fold difference in source strength embraced a percentile response from 16 to 84% and the precision of this response is indicated by the close replication in small groups of animals. The responses predicted by the Poisson law on the assumption that one organism is capable of initiating disease are 14 and 87 % versus 16 and 82 % observed.

In terms of original slurry dilutions, the level at which few or no responses occur and the level at which practically all animals respond to respiratory exposure are separated by an amount similar to that separating these same levels when exposure was by the intraperitoneal route. Thus, in both instances the findings are compatible with the belief that in the guinea pig a single particle inhaled or injected intraperitoneally is capable of initiating infection.

The response of guinea pig and man when exposed for similar periods simultaneously was then examined. The results (Table 2) show that clouds just capable of infecting most of the men exposed will infect only a small fraction of the guinea pigs exposed under identical conditions.

The results become even more interesting when it is remembered that the per minute respiratory exchange in man is some 100 times that of the guinea pig and that a 100-fold slurry dilution separates the levels at which 80% of men and of guinea pigs responded. Thus all of the data in the guinea pig, by intraperitoneal injection or by inhalation, and in man by inhalation fit a pattern compatible with initiation of infection by one organism.

Fuller (10) has employed a similar approach in the study of the infectious dose of *Rickettsia prowazekii*. Working with various dilutions of a rickettsial suspension, he showed that the amount required to infect a louse and the amount re-

TABLE 1. *Response of guinea pigs following respiratory exposure to Coxiella burnetii*

| Slurry dilution | Serological response in guinea pigs | |
|---|---|---|
| | Observed | Predicted |
| $10^{-6}$ | 0/16* | |
| | 0/13 | |
| $10^{-5}$ | 0/13 | (1.5%) |
| | 1/13 (4%) | |
| $10^{-4}$ | 2/13 | (14%) |
| | 2/11 (16%) | |
| $10^{-3}$ | 11/14 | (87%) |
| | 11/13 (82%) | |
| $10^{-2}$ | 14/14 | |
| | 14/14 | |

* Number infected/number exposed.

TABLE 2. *Response of man and guinea pigs simultaneously exposed to aerosols of Coxiella burnetii*

| Slurry dilution | Serological response in | |
|---|---|---|
| | Guinea pigs | Man |
| $10^{-6}$ | 0/29* | 0/2 |
| $10^{-5}$ | 1/26 | 4/5† |
| $10^{-4.5}$ | | 3/3 |
| $10^{-4}$ | 4/24 | 7/8 |
| $10^{-3}$ | 22/27 | 4/5 |
| $10^{-2}$ | 28/28 | 4/4 |
| $10^{-1}$ | 24/24 | 2/2 |

* Number infected/number exposed.
† Two inapparent infections.

quired to initiate disease in the cotton rat are closely comparable, if not identical.

Having considered the evidence that in Q fever one organism apparently can induce disease and that, at least in the guinea pig, route of administration over the dose range examined is not of particular importance, the effect of introducing multiples of this unit may now be examined. Figure 2 depicts the febrile pattern observed in adult guinea pigs inoculated intraperitoneally. Along the ordinates, plotted exponentially, are the dilutions of the slurry made prior to intra-

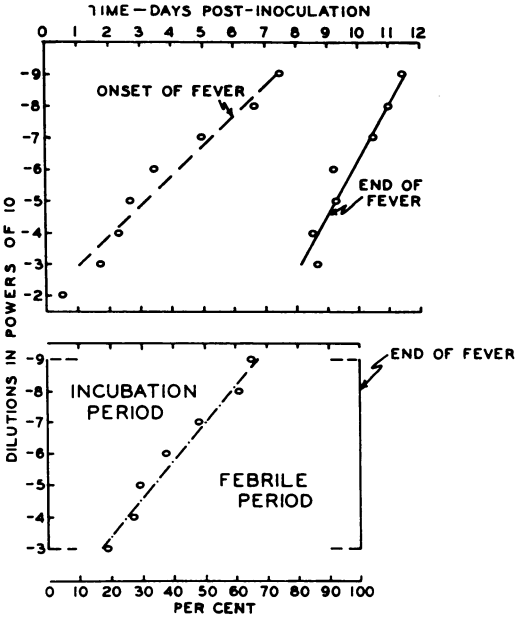


FIG. 2. *Top. Coxiella burnetii titration in guinea pigs. Intraperitoneal inoculation of WC-5; temperature readings q12h; 10 animals per point (all groups were febrile).*

*Bottom. Identical raw data as above, rearranged to show on percentage basis, the reciprocal relationship between incubation period and febrile period. -·-·- = (Period between inoculation and fever onset)/(period between inoculation and end of fever) × 100.*

peritoneal inoculation. Time in days is shown on the abscissas. Temperatures were taken every 12 hr in this particular titration and there were ten animals per group. The points given represent the arithmetical average of the time of appearance and of termination of fever at each dose level. Note that the incubation periods shorten as the dose increases and that the points are linear when plotted against the logarithms of dilutions of the slurry. When these animals are followed through to the end of their febrile period, a linear relationship is again apparent. The slopes are not identical so that with large doses the time between challenge and termination of fever is shorter than with small challenge doses. The lower part of Fig. 2 is simply a rearrangement to show the reciprocal relationship between incubation period and febrile period, the two being approximately equal at the mid-point of the titration.

This inverse dose-incubation period relation-

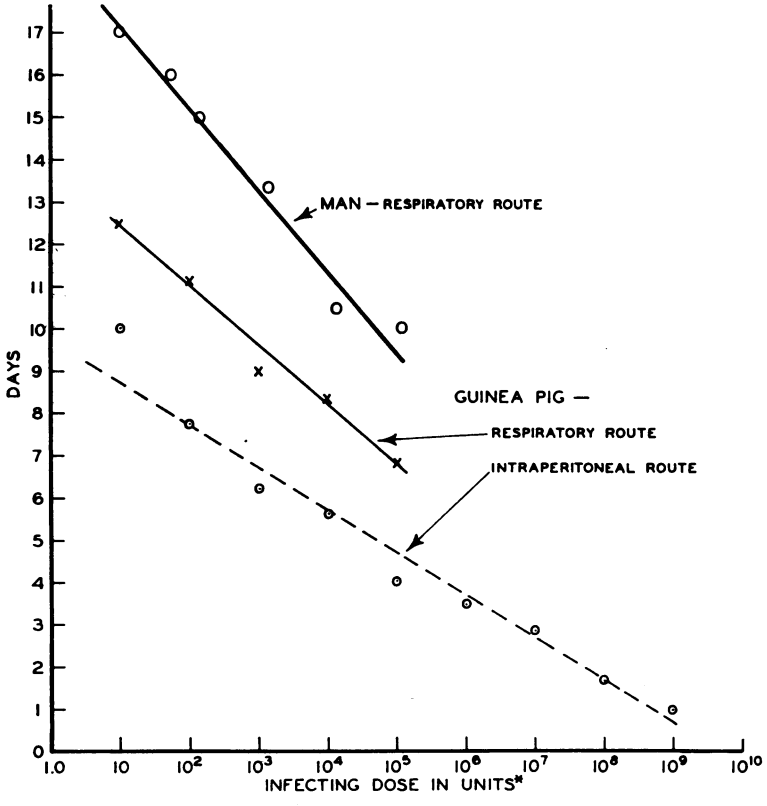


FIG. 3. *Average incubation periods with varying doses of Coxiella burnetii (AD strain).*

* One unit is that quantity of *C. burnetii* producing a serological response in 50% of exposed guinea pigs.

ship was examined in guinea pigs and men exposed by the respiratory route. The results show (Fig. 3) the similarity of the three regressions.

This dependence of the length of the incubation period on the size of the initial infecting dose is plainly consistent with the belief that the introduced rickettsiae behave independently and that manifestations of disease occur when the rickettsial burden attains a given level. It seems to make little difference whether the organisms multiply outside the body or within the body. In the guinea pig the intraperitoneal introduction of about 2 billion rickettsiae is associated with the prompt appearance of fever and this dosage also results in the death of most animals so injected. This number of organisms will also produce a prompt temporary fever in immune animals or in animals protected by previously administered antibiotics. With a very slight reduction in numbers to around $10^9$ organisms, no febrile response occurs if the material is injected into animals previously given broad spectrum antibiotics and continued on the drug for a period of 6 days thereafter; in such animals relapse does not occur when the drug is stopped and a solid immunity develops. Three days of a similar antibiotic regimen is not enough to accomplish this result and all animals will develop fever when the drug is stopped. Certain characteristics of the immune response in the guinea pig are then defined. A nearly fever-provoking mass of viable rickettsiae held in check for 6 days is sufficient to elicit a solid immune response without a requirement for significant multiplication.

It was assumed that a similar rickettsial burden occurred in man in the later part of his incubation period and that drug prophylaxis should abort the infection. As has been reported elsewhere (24), it was possible to suppress completely any clinical disease in man exposed by the respiratory route with a few days of oxytetracycline prophylaxis, providing administration of

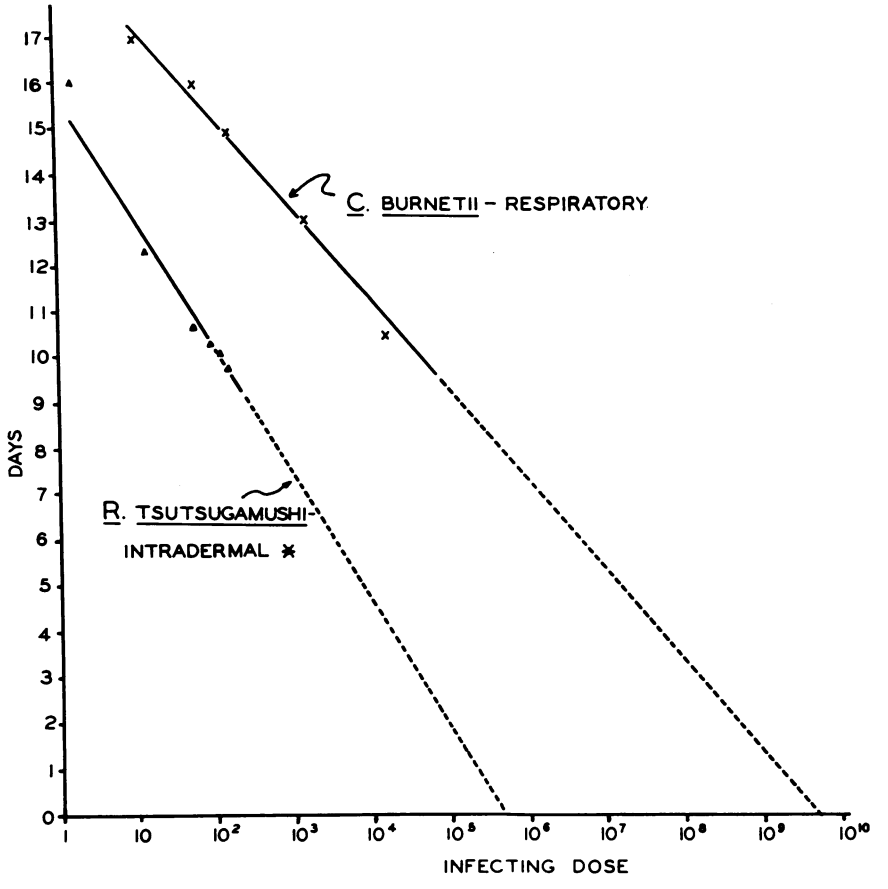


FIG. 4. *Average incubation periods in man with varying doses of Coxiella burnetii (AD strain) and Rickettsia tsutsugamushi (Karp strain).*

* Data after Ley et al. (14).

the drug was begun late in the incubation period. In these circumstances relapse did not occur.

In this respect, Q fever differs sharply from another of the rickettsial diseases, scrub typhus, in which relapses may occur even if therapy is begun after the onset of fever and in which relapses are expected if drug is used prophylactically (21). In both diseases data are available in man concerning the impact of the initial size of the infecting dose on the incubation period (14). These are summarized in Fig. 4. The solid lines connect the observed points. It will be remembered that in the Q fever guinea pig model the relationship between dose and incubation period was nearly linear over all dose ranges. Assuming that a similar linearity occurs in man, these observed values have been projected so as to cross the horizontal lower axis. This provides an approximation of the number of rickettsiae in man which should be sufficient, without significant multiplication, to induce fever. As drawn, there is almost a 10,000-fold difference predicted for the fever-producing mass of rickettsiae in the two diseases. If quantity of rickettsiae is as important in provoking an immune response in scrub typhus as it appears to be in Q fever, a reasonable explanation is afforded for success of late drug prophylaxis in the one and failure in the other.

There is a variety of other similarities between the responses observed in the guinea pig inoculated intraperitoneally, in the guinea pig infected by the respiratory route, and in man infected by inhalation. However, it is believed that sufficient examples have been given to indicate the value of the guinea pig for predicting the probable

course of this infection in man, particularly when certain points have been checked by direct comparison.

It is possible now to return to the original charge and examine the probable route taken by the airborne rickettsiae after their entry into the respiratory tract. For one student of Q fever the problem is very simple. Babudieri (4), referring to the disease in both man and animals, has the following on record: "It is now proved that if the virus has penetrated by inhalation there will be pulmonary localization of the disease and that there will not be pulmonary localization if *Coxiella* has infected the organism through food, by subcutaneous injection, or through the surface mucous membranes." Since the case fatality rate in man is very low, this has not been examined by histological approach. It can be examined in animals and here it is difficult to understand how Babudieri permitted himself such a rash statement. From the same period there are two reports from Italian workers (19, 20) concerning guinea pigs sacrificed on the first day of fever after intraperitoneal inoculation. Both authors record that pulmonary lesions were seen regularly. An adequate serial histological examination of guinea pigs after respiratory exposure has not been reported. Victor, Mika, and Goodlow (25) record "congestion" in the lungs of two animals examined 4 days after a small aerosol exposure. Based on our own casual observations in guinea pigs, subjected to respiratory and to intraperitoneal exposures with the AD strain and sacrificed at varying times thereafter, the time of appearance of the gross pulmonary lesions was similar. If other rickettsial infections in animals be included, attention should be given to the report by Okamoto (15) that following intraperitoneal inoculation of mice the rickettsia of endemic typhus could be demonstrated in the "alveolar" cells of the lung. In man the occurrence of pulmonary lesions in scrub typhus (2) and epidemic typhus (28) is well known. In both, of course, the initial route of infection is usually other than respiratory. Plainly then, a pulmonary route of infection is not necessary for the eventual development of lung lesions in the rickettsial diseases.

In many of the other diseases considered in this Conference there is little doubt that the introduction of a pathogen into the lungs frequently results in the development of a primary pulmonary lesion. Yet for airborne Q fever it is difficult to marshal any evidence that such an initial complex occurs, and, indeed, considerable circumstantial evidence can be advanced to indicate that the lungs serve mainly as a portal of direct entry. In its present form there is no reason to believe that *C. burnetii* is particularly well adapted for direct multiplication in the cells of the pulmonary tract. It seems worthwhile to examine these data and to give consideration to whether or not this airborne rickettsiosis differs in its initial pathogenesis from the bacterial and fungal infections.

In a common host of this disease, sheep, the intratracheal introduction of massive doses of California strains of *C. burnetii* usually produces neither overt disease nor the development of obvious pulmonary lesions (1). Such animals may circulate rickettsiae for a few days and then the organism becomes dormant only to undergo extensive multiplication in the placenta if at some future time the animal becomes pregnant (26). It appears therefore that in this normal disease cycle the production of pulmonary lesions is not essential.

A partially analogous situation seems to occur if men, rendered immune by vaccination, are exposed by the respiratory route to these organisms. Such men do not develop pulmonary lesions, yet the organism does enter the body and multiplies there to a level sufficient to provoke an antibody response. This has been seen in men exposed to respiratory doses of such a low level as to render a response without multiplication very unlikely. As supporting evidence for this statement, it may be noted that the time of appearance of circulating antibodies is also dose dependent, occurring about the time that nonimmunized men develop overt signs of illness.

Men who were given a prophylactic drug late in their incubation period did not develop pulmonary lesions; yet, as has been previously noted, they developed an immune response sufficient to prevent the appearance of fever after cessation of the drug. Further, men placed on drug after the onset of fever but who had no pulmonary lesions at the time oxytetracycline therapy was initiated did not, in any instance, develop discernible lesions later.

Approximately half of all of the cases of human Q fever, whether treated or not, do not show by roentgenographic examination any evidence of

pulmonary lesions. Yet in the experimental situation such individuals show circulating rickettsiae in their blood beginning sometimes as long as a week before the onset of fever, with a gradual increase in number as the incubation period progresses. The number of such organisms circulating in man is usually low since, except at the time of onset of actual disease, it is uncommon to evoke a serological response in all guinea pigs of a group, each of which has received 1 ml of blood from the patient. Yet there is clear evidence that a circulating rickettsemia preceded temporally the appearance of pulmonary lesions demonstrable by roentgenologic techniques.

In tularemia (16) and histoplasmosis (12) there is a direct relationship in man and in animals between the size of the inhaled dose of organisms and the number of primary pulmonary lesions which appear. Over a range in man varying from a minimal infectious dose to many thousand-fold, this dose relationship was not apparent for *C. burnetii*. A restriction has to be placed on this observation since in most instances in man the progress of the disease in the experimental situation was interrupted by chemotherapy, begun within 24 hr of the onset of fever. However, in a few untreated experimental cases and in numerous cases of this disease studied prior to the advent of chemotherapy, it has been uncommon to see more than three or four discrete pulmonary lesions in contrast to the multiplicity of lesions often seen following infection with bacteria or fungi.

It could be argued that of the numerous rickettsiae presented for inhalation only a very few take root in the lung and that these are the only effective organisms in the initiation of the infection. Such a hypothesis cannot be reconciled with the observations that, both in man and in animals infected by the respiratory route, the incubation period is obviously dose dependent.

In an effort to examine this problem in men, multiple blood cultures were taken during the several hours after respiratory infection. In one instance a rickettsial isolation was made from the blood drawn 4 hr after exposure. The theoretical presented respiratory dose for this man was 150 infectious units. Four hours is an extremely short interval for organisms to have passed through lymphatic channels and thus enter the blood stream. It seems necessary to assume a direct entry of at least some of the rickettsiae from the lung to the blood vascular system.

Some indirect support for failure of the lymphatic system to participate is provided by roentgenographic examinations. The radiographic picture of Q fever uncommonly includes obvious mediastinal or perihilar involvement, findings regularly observed in those diseases in which the primary route of spread in the body is via the lymphatics.

There is one report in which something approximating primary Q fever pneumonia in man is described. These are the three experimental cases of Blanc et al. (6), who employed a suspension, derived from ticks, which was said to be rich in rickettsiae. The method of exposure was to introduce this suspension into the nasal passages of the patient at the time he inspired deeply as the result of a convulsive electrical shock. The exact dosage was not reported but the onset of fever was within 5 days. Coincident with this there was described an "increase in the volume of the hilar shadows" followed in 2 or 3 days by evidence of pulmonary consolidation. Even then, with this massive dose introduced by rather unphysiological means directly into the lung, frank consolidation was not observed until 2 or 3 days after the onset of fever.

Mention should be made of the findings of Caminopetros (7), who inoculated sheep and goats by the intranasal route with the Balkan strain of *C. burnetii* (9). This resulted in fever and in pulmonary consolidation. The time of appearance of the pulmonary changes is not specified.

The introduction of large numbers of rickettsiae intranasally into anesthetized mice has been employed for the production of epidemic (8) and murine (11) typhus vaccines. After several passages to permit "adaptation," such a method of introduction results in gross pulmonary consolidation with early death. The number of pulmonary foci is directly correlated with the original dosage. Although this method of introduction of organisms into the lungs of rodents obviously differs markedly from the type of inhalation infection considered in this Conference, this known capability of certain rickettsiae to "adapt" for direct multiplication in cells of the respiratory tract deserves serious consideration. This probably was the basis for the following comment concerning Q fever made

by Stoker and Marmion (22): "The establishment of a variant which could multiply profusely and be excreted in large numbers from the respiratory rather than the reproductive tract might well lead to continued propagation of *R. burneti* in humans as an epidemic disease." To this we would add, as a final speculative note, that perhaps inherent differences in pneumotropism between various strains of *C. burnetii* could account for the reported high incidence of pulmonary lesions in some epidemics and the low incidence in others.

## Summary

Data have been reviewed which indicate that the infecting dose of the AD strain of *Coxiella burnetii* for man and guinea pig is similar and that the responses are such as to suggest that a single rickettsia is capable of initiating disease. With larger infecting doses the pattern is compatible with the belief that each infecting organism acts independently. Introduction by the intraperitoneal route or by inhalation in the guinea pig produces a similar type of disease. The guinea pig model, by either route, is closely similar to man infected by inhalation. Finally, the available evidence suggests that, in the model examined, primary multiplication at the site of initial contact in the lung probably is not important in disease initiation and certainly is of no importance so far as maintenance of the disease cycle is concerned. This is the situation as it exists today, yet for this comparatively simple and extensively studied disease it would appear to be necessary to end on a note of caution and to indicate that considerable further study may be needed. This attitude, which applies to all the deliberations of this Conference, has perhaps never been better expressed than by Popper (18) in his *Logic of Scientific Discovery* with this statement:

"Science does not rest upon rock-bottom. The structure of its theories rises, as it were, above a swamp. It is like a building erected on piles. The piles are driven down from above into the swamp, but not down to any natural or 'given' base; and when we cease our attempts to drive our piles into a deeper layer, it is not because we have reached firm ground. We simply stop when we are satisfied that they are firm enough to carry the structure, at least for the time being."

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